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# Screening for *UGT1A1* Genotype in Study A5257 Would Have Markedly Reduced Premature Discontinuation of Atazanavir for Hyperbilirubinemia

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**Background.** Some patients are not prescribed atazanavir because of concern about possible jaundice. Atazanavir-associated hyperbilirubinemia correlates with *UGT1A1* rs887829 genotype. We examined bilirubin-related discontinuation of atazanavir in participants from AIDS Clinical Trials Group Study A5257.

**Methods.** Discriminatory properties of *UGT1A1* T/T genotype for predicting bilirubin-related atazanavir discontinuation through 96 weeks after antiretroviral initiation were estimated.

**Results.** Genetic analyses involved 1450 participants, including 481 who initiated randomized atazanavir/ritonavir. Positive predictive values of rs887829 T/T for bilirubin-related discontinuation of atazanavir (with 95% confidence intervals [CIs]) were 20% (CI, 9%–36%) in Black, 60% (CI, 32%–84%) in White, and 29% (CI, 8%–58%) in Hispanic participants; negative predictive values were 97% (CI, 93%–99%), 95% (CI, 90%–98%), and 97% (CI, 90%–100%), respectively.

**Conclusions.** Bilirubin-related discontinuation of atazanavir was rare in participants not homozygous for rs887829 T/T, regardless of race or ethnicity. We hypothesize that the higher rate of discontinuation among White participants homozygous for rs887829 T/T may reflect differences in physical manifestations of jaundice by race and ethnicity. Selective avoidance of atazanavir initiation among individuals with T/T genotypes would markedly reduce the likelihood of bilirubin-related discontinuation of atazanavir while allowing atazanavir to be prescribed to the majority of individuals. This genetic association will also affect atazanavir/cobicistat.

**Keywords.** atazanavir; HIV; pharmacogenomics; pharmacokinetics; *UGT1A1*.

The once-daily human immunodeficiency virus (HIV)-1 protease inhibitor atazanavir with low-dose ritonavir as a pharmacokinetic enhancer (atazanavir/r) is generally safe and effective as a first-line regimen for HIV-1

infection [1–4]. Atazanavir increases plasma indirect bilirubin concentrations by inhibiting uridine diphosphate glucuronosyltransferase (UGT) 1A1-mediated bilirubin glucuronidation [5]. This provides a reliable biomarker of very recent medication adherence [6–8]. Atazanavir-associated indirect hyperbilirubinemia does not indicate hepatic injury [2, 9–11], but some patients discontinue atazanavir due to cosmetic jaundice [3, 12, 13], and many are not prescribed atazanavir to avoid this possibility. Better pretreatment prediction of atazanavir intolerance due to hyperbilirubinemia could reduce jaundice-related atazanavir discontinuations.

Polymorphisms in *UGT1A1* are associated with interindividual differences in plasma indirect bilirubin concentrations in the general population (ie, Gilbert's syndrome). A promoter tandem TA repeat, *UGT1A1*\*28

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(TA)<sub>7</sub>, is associated with reduced *UGT1A1* transcription compared with *UGT1A1*\*1 (TA)<sub>6</sub> [14, 15]. Among atazanavir recipients, *UGT1A1*\*28 has been strongly associated with unconjugated hyperbilirubinemia [16, 17], as has a C→T single-nucleotide polymorphism (rs887829) in almost complete linkage disequilibrium with *UGT1A1*\*28 [18]. In a genome-wide study involving individuals who had been randomized to atazanavir-containing regimens in AIDS Clinical Trials Group (ACTG) protocol A5202, rs887829 genotype, baseline indirect bilirubin, and baseline hemoglobin were each independently associated with peak on-treatment total bilirubin concentration [18]. However, reported associations between *UGT1A1* genotype and atazanavir discontinuation have been inconsistent [17, 19].

In ACTG protocol A5257, participants were randomized to receive atazanavir/r, darunavir/r (darunavir/r), or raltegravir, all with concomitant tenofovir disoproxil fumarate (TDF)/emtricitabine. The present study examined whether *UGT1A1* genotype, baseline indirect bilirubin, and/or baseline hemoglobin were associated with bilirubin-related discontinuation of atazanavir/r in A5257, and whether associations differed by race or ethnic group. We also assessed whether tolerability of regimens containing atazanavir/r compared with darunavir/r or raltegravir differed by *UGT1A1* genotype.

## METHODS

### Study Participants

Protocol A5257 was a phase III prospective randomized open-label trial comparing 3 nonnucleoside reverse transcriptase inhibitor-sparing antiretroviral regimens for initial treatment of HIV-1 infection. Primary results of A5257 were previously published [20]. In brief, A5257 participants enrolled from 2009 to 2011 at research sites in the United States and Puerto Rico were randomized to open-label atazanavir (300 mg) plus ritonavir (100 mg), darunavir (800 mg) plus ritonavir (100 mg), or raltegravir (400 mg), all with concomitant TDF/emtricitabine (300 mg/200 mg). Study medications were once daily except for raltegravir, which was twice daily. Study evaluations were performed before entry, at entry, at weeks 4, 8, 16 and 24, 36, and 48, and every 16 weeks thereafter until the last enrolled subject was followed for 96 weeks. Bilirubin and hemoglobin were assayed individually as per standard procedures at each clinical research site. Participants with asymptomatic hyperbilirubinemia were allowed to continue atazanavir/r if the site investigator felt that the hyperbilirubinemia was not clinically relevant.

### Identifying Genetic Polymorphisms

Consent for genetic analyses was obtained under ACTG protocol A5128 [21]. The Vanderbilt University Institutional Review Board and the ACTG approved this use of DNA. Genotypes for rs887829 (hereafter called *UGT1A1* genotype) were available

from Illumina HumanCore Exome Chip data generated by Vanderbilt Technologies for Advanced Genomics, and these genotypes provided results for 523 486 polymorphisms with call rates ≥99%. Laboratory personnel blinded to clinical data performed genotyping. Genetic data management and association analyses were performed with PLINK version 1.07 [22]. Samples that clustered to African American, European, or Hispanic genetic ancestry are hereafter called Black, White, and Hispanic, respectively. Clustering of samples was done by multidimensional scaling (MDS) implemented in PLINK.

### Statistical Analyses

The target population for this study was Black, White, or Hispanic participants who initiated randomized treatment in A5257 and had genotype data available. Primary endpoints for the analysis were time to bilirubin-related discontinuation of atazanavir/r by 96 weeks. Attribution of reasons for discontinuation of randomized treatment, including details of toxicity or adverse events and participant-initiated discontinuations that were due to low-grade adverse events, was based on site report. Bilirubin-related events were defined a priori as those reported as being due to jaundice, elevated bilirubin, or hyperpigmentation. Inconsistent or unclear information was reconciled by site query.

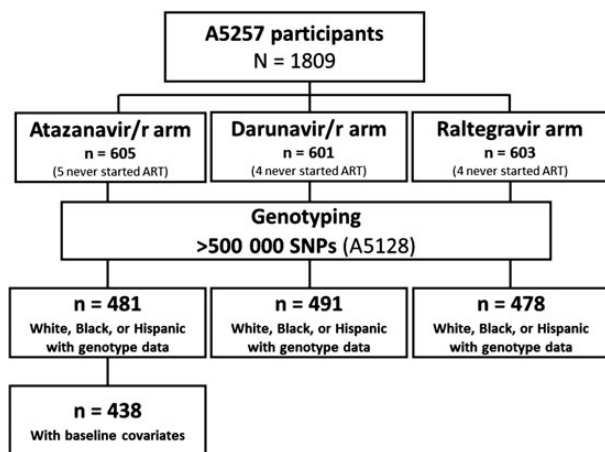
Cumulative incidence of bilirubin-related discontinuation of atazanavir/r was estimated using a competing risks framework and compared across *UGT1A1* genotypes using Gray's test. Cox proportional hazard models were used to estimate relative hazards of bilirubin-related discontinuation of atazanavir/r by *UGT1A1* genotype with and without adjusting for baseline indirect bilirubin and baseline hemoglobin. Mirroring the primary A5257 analyses, the cumulative incidence of tolerability failure over 96 weeks was estimated by *UGT1A1* genotype with pairwise differences compared between randomized atazanavir/r, darunavir/r, and raltegravir via 97.5% confidence intervals (CIs) [20]. Discriminatory properties (positive predictive value [PPV] and negative predictive value [NPV]) of *UGT1A1* T/T genotype for predicting bilirubin-related atazanavir/r discontinuation through 96 weeks after antiretroviral initiation (binary outcome) were estimated with exact 95% CIs. All analyses were performed using SAS 9.2 without adjusting for multiple comparisons.

All analyses were done separately by self-identified race or ethnicity. To minimize potential errors due to race or ethnicity misclassification, sensitivity analyses were performed based on inferred ancestry using genome-wide data with exclusion of samples that did not cluster with African American, European, or Hispanic populations.

## RESULTS

### Study Participants

Among 605 participants randomized to the atazanavir/r-containing arm in A5257, 600 initiated the regimen and had



**Figure 1.** Derivation of the study population. Of 605 participants randomized to the atazanavir/r arm in A5257, a total of 481 were included in genetic association analyses for treatment discontinuation and 438 in analyses with baseline covariates. Of participants randomized to the darunavir/r and raltegravir arms, 491 and 478, respectively, were included in analyses for treatment discontinuation. Abbreviations: ART, antiretroviral therapy; SNP, single-nucleotide polymorphism.

available treatment discontinuation data. Among these 600 participants, 493 participants had genotype data available, 481 of whom were self-identified as Black non-Hispanic, White non-Hispanic, or Hispanic. Derivation of the study population is shown in Figure 1. Baseline characteristics and genotype frequencies of study participants from the atazanavir/r arm are shown in Table 1. Among the 481 participants, median age was 38 years, median body mass index was 25 kg/m<sup>2</sup>, and 23% were female, consistent with the full study population. Frequency of *UGT1A1* T/T genotype varied by race or ethnicity (19% in Black, 8% in White, 16% in Hispanic participants). Genotype data were unavailable on 107 participants because they either did not consent to genetic testing or because genotyping was unsuccessful.

### Associations With Bilirubin-Related Atazanavir/r Discontinuation

Among the 481 evaluable participants, 146 (30%) prematurely discontinued atazanavir/r (29%, 30%, and 34% among Black, White, and Hispanic participants, respectively). Among these 146 participants, most atazanavir/r discontinuations were not bilirubin-related, regardless of race or ethnicity; discontinuation was considered bilirubin-related in 37 (25%), including 13 (21%) of 62 Black participants, 18 (33%) of 54 White participants, and 6 (20%) of 30 Hispanic participants. Among all 481 participants, there was an 8% likelihood of bilirubin-related discontinuation of atazanavir/r (6%, 10%, and 7% among Black, White, and Hispanic participants, respectively). Other frequently reported reasons of atazanavir/r discontinuation were nonadherence (n = 35) and gastrointestinal toxicity (n = 21).

**Table 1. Baseline Characteristics of Study Participants**

	Drug Discontinuation Analyses (N = 481)
Race/ethnicity	
Black, n (%)	211 (44%)
<i>UGT1A1</i>	
C/C	70 (33%)
C/T	101 (48%)
T/T	40 (19%)
White, n (%)	183 (38%)
<i>UGT1A1</i>	
C/C	83 (45%)
C/T	85 (46%)
T/T	15 (8%)
Hispanic, n (%)	87 (18%)
<i>UGT1A1</i>	
C/C	31 (36%)
C/T	42 (48%)
T/T	14 (16%)
Female	112 (23%)
Median BMI in kg/m <sup>2</sup> (IQR)	25 (23–29)
Median age in years (IQR)	38 (29–45)
Median baseline bilirubin mg/dL (IQR)	0.4 (0.2–0.6) <sup>a</sup>
Median baseline hemoglobin g/dL (IQR)	13.9 (12.4–14.9) <sup>a</sup>

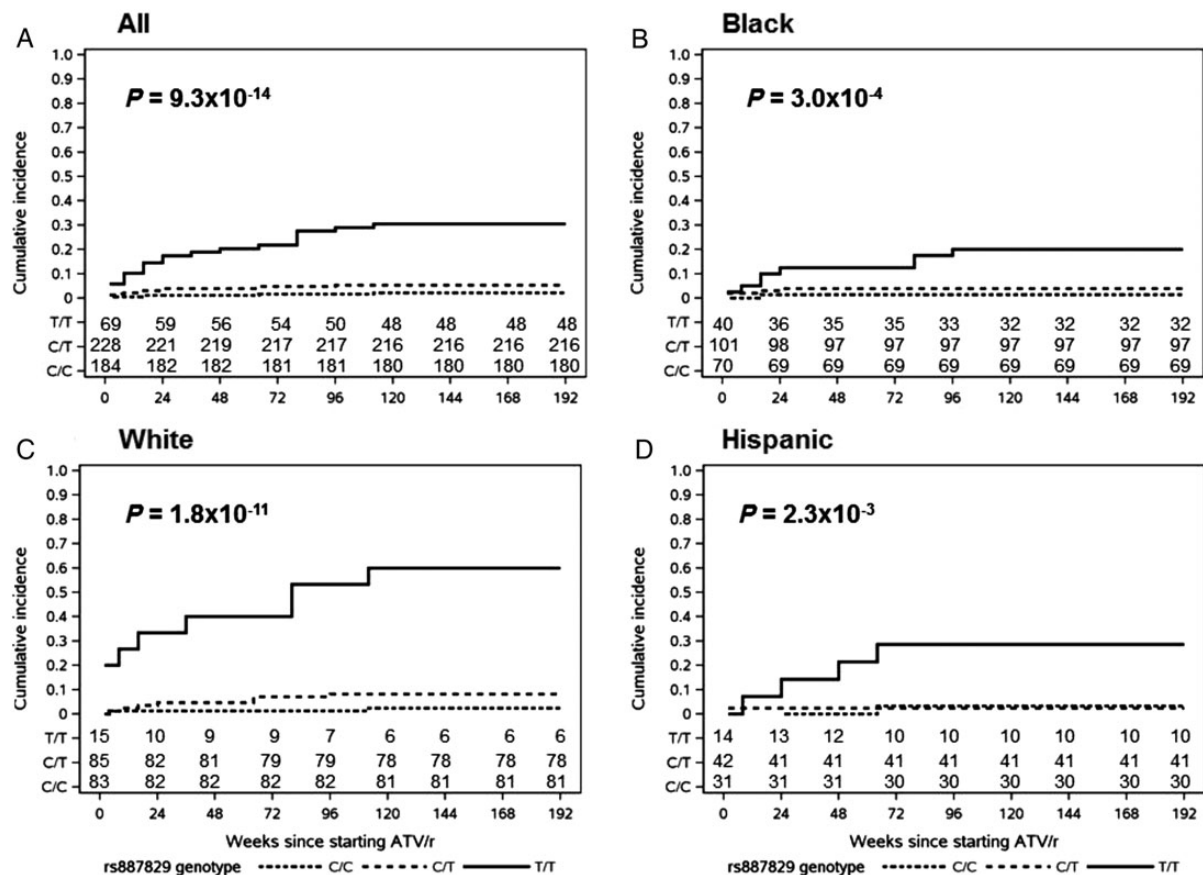
Abbreviations: BMI, body mass index; IQR, interquartile range.

<sup>a</sup> Based on 438 participants with baseline covariates.

The *UGT1A1* genotype was associated with time to bilirubin-related discontinuation of atazanavir/r in Black ( $P = 3.0 \times 10^{-4}$ ), White ( $P = 1.8 \times 10^{-11}$ ), and Hispanic ( $P = 2.3 \times 10^{-3}$ ) participants (Figure 2). Participants with T/T genotype had higher cumulative incidence of bilirubin-related atazanavir/r discontinuation compared with non-TT genotypes regardless of race or ethnicity by visual inspection; the magnitude of the effect varied by race or ethnicity ( $P < .0001$ ). In Cox proportional hazards models stratified by race/ethnicity, and that included baseline indirect bilirubin and baseline hemoglobin, only *UGT1A1* T/T genotype was independently associated with bilirubin-related atazanavir/r discontinuation, with a hazard ratio that ranged from 10 in Black participants to 24 in White participants (Table 2).

### Discriminative Properties of Bilirubin-Related Discontinuation of Atazanavir

Positive and NPVs of *UGT1A1* genotype and/or baseline indirect bilirubin for bilirubin-related discontinuation of atazanavir/r by 96 weeks are presented in Table 3. Among participants with *UGT1A1* T/T genotypes, the probabilities of bilirubin-related discontinuation of atazanavir/r (ie, PPV) were 20% in Blacks, 60% in Whites, and 29% in Hispanics. Among participants with non-TT *UGT1A1* genotypes, the



**Figure 2.** Cumulative incidence of time to bilirubin-associated discontinuation of atazanavir/r stratified by *UGT1A1* genotype. Lines estimate the cumulative incidence of time to bilirubin-associated discontinuation of atazanavir, stratified by *UGT1A1* rs887829 genotype and self-reported race or ethnicity. (A) All participants; (B) Black participants; (C) White participants; (D) Hispanic participants. *P* values are given by Gray's test for testing equality of cumulative incidence functions. Solid lines represent rs887829 T/T, dashed lines represent rs887829 C/T, and dotted lines represent rs887829 C/C. Number of participants in the risk set over time for each genotype are shown in each plot.

probability of not experiencing bilirubin-related discontinuation of atazanavir/r (ie, NPV) ranged from 95% to 97%.

The PPV of baseline indirect bilirubin >0.4 mg/dL (threshold based on the median value) for bilirubin-related discontinuation of atazanavir/r by 96 weeks stratified by race or ethnicity ranged from 13% to 18%, whereas the NPV ranged from 96% to 98%. Considering the combination of *UGT1A1* T/T genotype and baseline indirect bilirubin >0.4 mg/dL, by race or ethnicity, the PPV ranged from 23% to 75%, and the NPV ranged from 94% to 97%.

### Comparisons Between Randomized Treatment Arms

In A5257, primary treatment arm comparisons for all-cause tolerability failure over 96 weeks showed atazanavir/r to be inferior to both darunavir/r and raltegravir [20]. We repeated these analyses, within subgroups defined by *UGT1A1* genotype. Comparing atazanavir/r and darunavir/r arms, among participants with *UGT1A1* C/C, the estimated difference in 96-week cumulative incidence of all-cause tolerability failure was within the

prespecified A5257 equivalence boundary, and among participants with *UGT1A1* C/T was close to this boundary. In contrast, the estimated differences in 96-week cumulative incidence of tolerability failure with 97.5% CIs demonstrated inferiority for atazanavir/r compared with raltegravir regardless of *UGT1A1* genotype (Figure 3).

### Sensitivity Analyses

Sensitivity analyses were performed based on inferred ancestry using genome-wide data. These analyses censored 28 participants who, by MDS plot, did not cluster into distinct subgroups. Results and conclusions were unchanged in sensitivity analyses after removal of outliers (data not shown).

## DISCUSSION

The present study showed that, among 481 participants who initiated atazanavir/r in A5257, the likelihood of bilirubin-related discontinuation of atazanavir/r was low among participants

**Table 2. Cox Proportional Hazard Model for Bilirubin-Associated Discontinuation of Atazanavir/r With *UGT1A1* Genotype, Baseline Indirect Bilirubin, and Baseline Hemoglobin**

	Estimate (SE)	HR [95% CI]	P Value
<b>Black</b>			
rs887829 T/T	2.32 (1.09)	10 [1.2, 85]	.033
rs887829 C/T	0.94 (1.12)	2.6 [.3, 23]	.40
Baseline indirect bilirubin	0.83 (0.61)	2.3 [.7, 7.6]	.17
Baseline hemoglobin	0.57 (0.56)	1.8 [.6, 5.4]	.31
<b>White</b>			
rs887829 T/T	3.16 (0.83)	24 [4.7, 119]	$1.3 \times 10^{-4}$
rs887829 C/T	1.02 (0.81)	2.8 [.6, 14]	.21
Baseline indirect bilirubin	1.05 (0.63)	2.9 [.8, 9.9]	.09
Baseline hemoglobin	−0.04 (0.52)	1.3 [.3, 2.7]	.93
<b>Hispanic</b>			
rs887829 T/T	2.48 (1.15)	12 [1.3, 113]	.031
rs887829 C/T	−0.27 (1.43)	0.8 [.0, 13]	.85
Baseline indirect bilirubin	0.39 (0.91)	1.5 [.3, 8.8]	.66
Baseline hemoglobin	1.62 (1.20)	5.1 [.5, 53]	.17

Abbreviations: CI, confidence interval; HR, hazards ratio; SE, standard error.

with *UGT1A1* non-T/T genotypes (ie, C/C or C/T) regardless of race or ethnicity, and this was substantially higher among participants with *UGT1A1* T/T genotype regardless of race or ethnicity and was particularly high among White participants. These findings indicate that avoidance of atazanavir prescribing to individuals with *UGT1A1* T/T genotypes would have reduced

by approximately one-half the likelihood of bilirubin-related discontinuation of atazanavir, regardless of race or ethnicity. This approach would have still allowed atazanavir/r to be prescribed to the large percentage of individuals (81% of Black participants, 92% of White participants, and 84% of Hispanic participants) who are at low risk for bilirubin-related discontinuation.

We also examined the ability of baseline indirect bilirubin to predict bilirubin-related discontinuation of atazanavir among 438 participants who initiated atazanavir/r and had available baseline indirect bilirubin, regardless of genotype. Selective avoidance of atazanavir/r in individuals with baseline indirect bilirubin >0.4 mg/dL would have also reduced by approximately one half the likelihood of bilirubin-related discontinuation of atazanavir, regardless of race or ethnicity, but would have allowed atazanavir/r to be prescribed to only 70% of Black participants, 55% of White participants, and 65% of Hispanic participants.

Finally, we examined the predictive utility of *UGT1A1* genotype and baseline indirect bilirubin in combination. With both *UGT1A1* T/T genotype and baseline indirect bilirubin >0.4 mg/dL, the likelihood of bilirubin-related discontinuation of atazanavir/r was modestly higher compared with considering baseline indirect bilirubin or *UGT1A1* genotype alone. Prescribing atazanavir/r only to individuals who both lacked *UGT1A1* T/T and had baseline indirect bilirubin ≤0.4 mg/dL would have also reduced by approximately one half the likelihood of bilirubin-related discontinuation of atazanavir, regardless of race or ethnicity, but would have allowed atazanavir/r prescribing to only 62% of Black participants, 53% of White participants, and 57% of Hispanic participants.

Overall, the reductions in bilirubin-related discontinuation were similar in all race and ethnic groups whether by screening

**Table 3. Positive and Negative Predictive Values of *UGT1A1* rs887829 and Baseline Bilirubin for Bilirubin-Associated Drug Discontinuation**

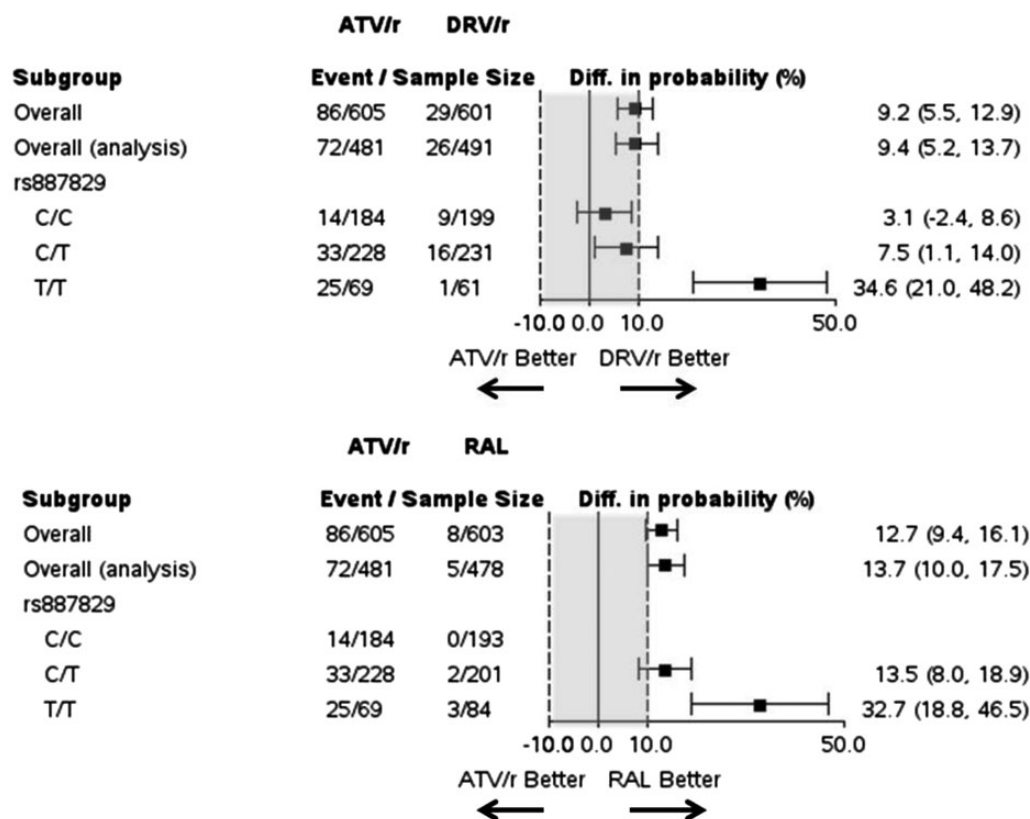
	Positive Predictive Value <sup>a</sup> [No. of Subjects Positive Test] (95% CI)	Negative Predictive Value <sup>b</sup> [No. of Subjects Negative Test] (95% CI)	Percentage That Would Not Be Prescribed Atazanavir/r if Screened
<b><i>UGT1A1</i> T/T genotype</b>			
Black	20% [40] (9%–36%)	97% [171] (93%–99%)	19.0%
White	60% [15] (32%–84%)	95% [168] (90%–98%)	8.2%
Hispanic	29% [14] (8%–58%)	97% [73] (90%–100%)	16.1%
<b>Baseline bilirubin</b>			
Black	14% [59] (6%–25%)	96% [139] (92%–99%)	29.8%
White	18% [78] (10%–28%)	96% [94] (89%–99%)	45.3%
Hispanic	13% [24] (3%–32%)	98% [44] (88%–100%)	35.3%
<b><i>UGT1A1</i> genotype and baseline bilirubin</b>			
Black	23% [22] (8%–45%)	98% [123] (94%–100%)	37.9%
White	75% [12] (43%–95%)	96% [91] (89%–99%)	47.1%
Hispanic	40% [5] (5%–85%)	97% [39] (87%–100%)	42.6%

Abbreviation: CI, confidence interval.

<sup>a</sup> Positive test: T/T genotype, or baseline bilirubin >0.4 mg/dL, or T/T genotype and baseline bilirubin >0.4 mg/dL.

<sup>b</sup> Negative test: non-T/T genotype, or baseline bilirubin ≤0.4 mg/dL, or non-T/T genotype and baseline bilirubin ≤0.4 mg/dL.





**Figure 3.** Pairwise treatment differences in cumulative probability of all-cause tolerability failure by 96 weeks. (Top) Comparison between atazanavir/r and darunavir/r arms. (Bottom) Comparison between atazanavir/r and raltegravir arms. Within each treatment comparison, the following are shown: Overall, primary result from ACTG A5257; Overall (analysis), result restricted to participants with *UGT1A1* genotype data; rs887829, results stratified by *UGT1A1* genotype. Estimates of pairwise treatment differences are shown with 97.5% confidence interval. The equivalence boundary for treatment comparison is  $\pm 10\%$  and is represented by the shaded area. Abbreviations: ATV, atazanavir; DRV, darunavir; RAL, raltegravir.

for low risk based on baseline indirect bilirubin  $< 0.4$ , based on non-TT *UGT1A1* genotype, or considering both. However, considering just *UGT1A1* genotype would allow atazanavir/r prescribing a substantially greater percentage of participants. In particular, among White participants, 92% could be prescribed to atazanavir/r if screened using *UGT1A1* genotype compared with only 55% if screened using baseline indirect bilirubin.

With *UGT1A1* T/T homozygosity, the likelihood of bilirubin-related atazanavir/r discontinuation was least in Black participants, intermediate in Hispanic participants, and greatest in White participants. We speculate that the extent to which jaundice becomes visible differs depending on skin color and/or ancestry.

Two previous studies that analyzed associations between *UGT1A1* genotype used all-cause discontinuation of atazanavir/r as the outcome of interest. In an observational study involving 121 Swiss HIV Cohort Study participants (80% Caucasian) who had received atazanavir/r, carriage of *UGT1A1* low expresser alleles (\*28/\*28 or \*28/\*37) was associated with increased risk of atazanavir/r discontinuation, with cumulative

rates of 63% among 18 participants carrying 2 alleles, 24% among 48 participants carrying one allele, and 15% among 55 participants carrying no allele [19]. (The \*28 and \*37 low-expresser alleles and the \*36 high-expresser allele of *UGT1A1* are all collectively identified as rs8175347.) In contrast, an analysis involving 646 participants randomized to receive atazanavir/r in ACTG protocol A5202 found no significant association between low-expresser *UGT1A1* genotype (primarily *UGT1A1*\*28) and increased likelihood of atazanavir/r discontinuation among either White participants or Black participants, although there was an association among Hispanic participants [17]. Our use of bilirubin-related atazanavir/r discontinuation as the outcome minimized confounding by other factors unrelated to *UGT1A1* genotype (eg, nonadherence) and allowed genotype-phenotype associations to be more precisely described. The somewhat different results of these 3 studies are not due to genotyping differences (rs8175347 in prior studies, rs887829 in the present study). These polymorphisms are in almost complete linkage disequilibrium (rs887829 C with both \*1 and \*36; rs887829 T with both \*28 and \*37), and the \*36 and

\*37 alleles of rs8175347 are infrequent in the populations studied.

There are several possible explanations for different results between the present study and prior reports. The somewhat higher atazanavir/r discontinuation rates in the Swiss HIV Cohort Study (including those not homozygous for the risk allele) might have been influenced by discontinuations that were unrelated to bilirubin. The threshold to discontinue atazanavir/r for jaundice might have been lower in that observational study than in A5257, a prospective clinical trial. Likewise, in ACTG study A5202, atazanavir/r discontinuations unrelated to bilirubin might have obscured associations with *UGT1A1* genotype. Consistent with this finding, most atazanavir/r discontinuations in the present study were attributed to causes other than bilirubin. In the A5202 study, the alternative antiretroviral provided for participants who developed jaundice (ie, lopinavir/r) may have been considered less attractive than the alternatives provided by protocol A5257 (ie, darunavir/r and raltegravir), creating a greater barrier to atazanavir/r discontinuation in A5202.

The HIV-1 protease inhibitors have relatively high genetic barriers to viral drug resistance. For this reason, protease inhibitor-based regimens are sometimes prescribed to patients who are at risk for nonadherence. With atazanavir/r, absence of an increase in plasma bilirubin from baseline (regardless of *UGT1A1* genotype) strongly indicates that atazanavir/r was not taken within the prior 24 hours. This biomarker of adherence, often available from chemistry panels obtained at routine clinic visits, could still be used among individuals with *UGT1A1* non-TT genotypes who are prescribed atazanavir/r. Prior cost-effectiveness modeling indicated that *UGT1A1* genotyping to avoid atazanavir-related hyperbilirubinemia is cost effective if comparator antiretroviral therapy regimens (eg, atazanavir/r versus darunavir/r, each with TDF/emtricitabine) have identical drug costs [23]. With *UGT1A1* genotyping for atazanavir, drug cost largely drives overall cost effectiveness. In settings where differential drug costs encourage prescribing of atazanavir/r-based regimens, patient care may benefit from *UGT1A1* genotyping, with avoidance of atazanavir in the select subset of patients at greatest risk for bilirubin-related discontinuation. Many commercial laboratories in the United States offer genotype testing for *UGT1A1* (especially *UGT1A1*\*28, which is in almost complete linkage disequilibrium with rs887829).

Among A5257 participants with *UGT1A1* C/C genotype, the cumulative incidence of all-cause tolerability failure by 96 weeks with atazanavir/r versus darunavir/r was within the equivalence boundary, and tolerability failure among participants with *UGT1A1* C/T genotype was almost within the equivalence boundary. The tolerability benefit of darunavir/r over atazanavir/r in A5257 was most pronounced among participants who were homozygous for *UGT1A1* T/T genotype. For atazanavir/r versus raltegravir comparisons, the cumulative incidence of all-

cause tolerability failure by 96 weeks was inferior in atazanavir/r even when considering the *UGT1A1* genotype.

The present study had limitations. We focused on genotype and clinical laboratory factors that were predetermined based on previous studies [17, 19]. It is possible that additional genetic or clinical factors could improve prediction of bilirubin-associated discontinuation of atazanavir/r. The smaller sample size for Hispanic participants may limit generalizability of findings in this subgroup. We did not study atazanavir with pharmacokinetic enhancement by cobicistat rather than ritonavir. However, atazanavir plasma concentration-time profiles when prescribed with cobicistat are bioequivalent to those with ritonavir [24]. Furthermore, in a double-blind clinical trial that randomly assigned 692 patients to receive atazanavir with either cobicistat or ritonavir, adverse events related to bilirubin elevations (eg, hyperbilirubinemia, jaundice, and scleral icterus) occurred in a similar percentage of patients in the cobicistat and ritonavir arms (40.7% and 36.2%, respectively), as did bilirubin-associated discontinuation of atazanavir (3.5% and 3.2%, respectively) [25]. We therefore expect the associations between *UGT1A1* genotype and bilirubin-associated discontinuation of atazanavir/r reported in the present study to also occur with atazanavir/cobicistat.

## CONCLUSIONS

In summary, among participants randomized to receive atazanavir/r with concomitant TDF/emtricitabine in A5257, *UGT1A1* T/T genotype predicted an increased likelihood of bilirubin-associated discontinuation of atazanavir/r, regardless of race or ethnicity. Selective avoidance of atazanavir initiation among individuals with *UGT1A1* T/T genotype would markedly reduce the likelihood of bilirubin-related discontinuation of atazanavir/r for hyperbilirubinemia. On April 8, 2015, the Department of Health and Human Services' Antiretroviral Guidelines for Adults and Adolescents were updated to move atazanavir from preferred to alternative status, explicitly based on results of A5257 [4]. The present analyses suggest that, among individuals known to have either rs887829 C/C or C/T genotypes, atazanavir might still be considered for inclusion in preferred regimens.

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## References

- Gatell J, Salmon-Ceron D, Lazzarin A, et al. Efficacy and safety of atazanavir-based highly active antiretroviral therapy in patients with virologic suppression switched from a stable, boosted or unboosted protease inhibitor treatment regimen: the SWAN Study (A1424-097) 48-week results. *Clin Infect Dis* **2007**; 44:1484–92.
- Molina JM, Andrade-Villanueva J, Echevarria J, et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naïve HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. *Lancet* **2008**; 372:646–55.
- Daar ES, Tierney C, Fischl MA, et al. Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. *Ann Intern Med* **2011**; 154:445–56.
- Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Available at: <http://www.aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf>. Accessed 19 May 2015.
- Zhang D, Chando TJ, Everett DW, et al. In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab Dispos* **2005**; 33:1729–39.
- Petersen K, Riddle MS, Jones LE, et al. Use of bilirubin as a marker of adherence to atazanavir-based antiretroviral therapy. *AIDS* **2005**; 19:1700–2.
- Rekic D, Clewe O, Roshammar D, et al. Bilirubin—a potential marker of drug exposure in atazanavir-based antiretroviral therapy. *AAPS J* **2011**; 13:598–605.
- Morello J, Alvarez E, Cuenca L, et al. Short communication: use of serum bilirubin levels as surrogate marker of early virological response to atazanavir-based antiretroviral therapy. *AIDS Res Human Retrovirus* **2011**; 27:1043–5.
- Malan DR, Krantz E, David N, et al. Efficacy and safety of atazanavir, with or without ritonavir, as part of once-daily highly active antiretroviral therapy regimens in antiretroviral-naïve patients. *J Acquir Immune Defic Syndr* **2008**; 47:161–7.
- Cleijns RM, van de Ende ME, Kroon FP, et al. Therapeutic drug monitoring of the HIV protease inhibitor atazanavir in clinical practice. *J Antimicrob Chemother* **2007**; 60:897–900.
- Torti C, Lapadula G, Antinori A, et al. Hyperbilirubinemia during atazanavir treatment in 2,404 patients in the Italian atazanavir expanded access program and MASTER Cohorts. *Infection* **2009**; 37:244–9.
- Puls R, Srasuebku P, Petoumenos K, et al. Efavirenz versus boosted atazanavir or zidovudine and abacavir in antiretroviral treatment-naïve, HIV-infected subjects: week 48 data from the Altair study. *Clin Infect Dis* **2010**; 51:855–64.
- McDonald C, Uy J, Hu W, et al. Clinical significance of hyperbilirubinemia among HIV-1-infected patients treated with atazanavir/ritonavir through 96 weeks in the CASTLE study. *AIDS Patient Care STDS* **2012**; 26:259–64.
- Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *New Engl J Med* **1995**; 333:1171–5.
- Monaghan G, Ryan M, Seddon R, et al. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* **1996**; 347:578–81.
- Rotger M, Taffe P, Bleiber G, et al. Gilbert syndrome and the development of antiretroviral therapy-associated hyperbilirubinemia. *J Infect Dis* **2005**; 192:1381–6.
- Ribaud HJ, Daar ES, Tierney C, et al. Impact of UGT1A1 Gilbert variant on discontinuation of ritonavir-boosted atazanavir in AIDS Clinical Trials Group Study A5202. *J Infect Dis* **2013**; 207:420–5.
- Johnson DH, Venuto C, Ritchie MD, et al. Genomewide association study of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group protocol A5202. *Pharmacogenet Genomics* **2014**; 24:195–203.
- Lubomirov R, Colombo S, diJulio J, et al. Association of pharmacogenetic markers with premature discontinuation of first-line anti-HIV therapy: an observational cohort study. *J Infect Dis* **2011**; 203:246–57.
- Lennox JL, Landovitz RJ, Ribaud HJ, et al. Efficacy and tolerability of 3 nonnucleoside reverse transcriptase inhibitor-sparing antiretroviral regimens for treatment-naïve volunteers infected with HIV-1: a randomized, controlled equivalence trial. *Ann Intern Med* **2014**; 161:461–71.
- Haas DW, Wilkinson GR, Kuritzkes DR, et al. A multi-investigator/institutional DNA bank for AIDS-related human genetic studies: AACTG Protocol A5128. *HIV Clin Trials* **2003**; 4:287–300.
- Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **2007**; 81:559–75.
- Schackman BR, Haas DW, Becker JE, et al. Cost-effectiveness analysis of UGT1A1 genetic testing to inform antiretroviral prescribing in HIV disease. *Antivir Ther* **2013**; 18:399–408.
- Ramanathan S, Warren D, Wei L, Kearney BP. Pharmacokinetic boosting of atazanavir with the pharmacoenhancer GS-9350 versus ritonavir. In: abstract of 49th Interscience Conference on Antimicrobial Agents and Chemotherapy. 2009, September 12–15, San Francisco, CA. Abstract 614.
- Gallant JE, Koenig E, Andrade-Villanueva J, et al. Cobicistat versus ritonavir as a pharmacoenhancer of atazanavir plus emtricitabine/tenofovir disoproxil fumarate in treatment-naïve HIV type 1-infected patients: week 48 results. *J Infect Dis* **2013**; 208:32–9.